STUDIES ON BACILLUS TYPHOSUS: A COMPARISON OF THEIR CULTURAL AND SEROLOGICAL RELATIONS.

by

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STUDIES ON BACILLUS TYPHOSUS: A COMPARISON OF THEIR CULTURAL AND SEROLOGICAL RELATIONS.

During the course of routine diagnostic work in this laboratory it was observed that a few organisms which were culturally typhoid were not agglutinated by some of the immune sera used for identification.

Antigenic differences in Bacillus typhosus have been noted by several observers. It has long been known that freshly isolated strains may become agglutinable only after long cultivation on artificial media. Stober[1] worked with a strain isolated from urine which did not agglutinate with serum from three cases of typhoid and agglutinated in low dilutions only with serum from seven other cases. This property had persist-ed for fourteen months. Moon[2] had also noted antigenic differences. In 1917 Weiss[3] published a tentative typing of typhoid based on cultural and antigenic differences. We were able to confirm Weiss's report to a great extent.

Technic: Twenty-five strains of typhoid were collected from as widely separated sources as possible. The source, place of isolation, name and date are tabulated in Table I.

Table I.

No.	Source	Name	Date
1	Blood culture, Lawrence Ks.	57	1913
21	" " Kansas City Mo.		1919
23	" " Univ. of Calif.		1914
25	" " Johns Hopkins Hospital		
4	Feces, Lawrence Ks.	Smith	1919
5	" " "	Hunter	1919
6	" " "		1918
8	" " "		1919
16	" Carrier, Beau Desert, France.		1918
20	" Topeka Ks.		1919
24	" Fatal case, Johns Hopkins.		1919
7	Spinal fluid, Halstead Ks.		1919
12	Spleen, Autopsy.	Rawlings	
18	" "	"	
15	Gallbladder, Autopsy, France.	Wable	1918
2	No history, N.Y. Board of Health		
3	" " " " City Board of Health	Bender	
10	" " " " " " "	Mt Sinai	
11	" " " " " " "	Pfeiffer	
13	" " American Museum	Hopkins	
14	" " " "	Miller	
17	" " Institute of Berlin.	Ebert	1888
19	" " Univ. of Chicago.	Jordan	1889
26	" " Johns Hopkins Hospital.		

Before making any serological tests it was considered advisable to run all strains through various culture media. The carbohydrate media used was semi-solid to which was added 1% of the carbohydrate desired and Andrade indicator to make a pale flesh color when cold. For the lead acetate agar 1cc of a 1% lead acetate solution was added to semisolid. The cultural reactions are tabulated in Table II.

It will be observed that the various media used by me differ in some respects from that recommended by the Journal of Bacteriology[4]. On account of the difficulty experienced in obtaining some of the carbohydrates it was necessary to make these changes.

In litmus milk all strains showed an initial acidity in twenty-four hours, fourteen strains remained acid after three weeks, seven strains became neutral after three weeks, three strains neutral after one week and one strain #5 became neutral after forty-eight hours and strongly alkaline after one week but never showed any peptonization.

In 2% peptone gelatine,(made according to the formula devised by Treece*), all strains except #5 and #7 gave a greenish black cloud around the stab.

* Unpublished paper, A Substitute for Adonite in the Determination of Fecal and Non-Fecal Strains in the Colon-Aerogenes Group.

TABLE II.

Strains	Motility	Gram	2% Gelatine	Indol	Lead Acetate	Xylose	Arabinose	Dulcite	Salacin	Dextrose	Mannite	Maltose	Lactose	Saccharose	Litmus Milk: 24 hours	Litmus Milk: 48 hours	Litmus Milk: 1 week	Litmus Milk: 3 weeks
1																	N	
2																	N	
3																	N	
4																		
5	I+					Negative										N	A	A
6																		
7																		N
8																		
9									Ac									
10																		
11																		
12						Negative	Negative			Positive								
13								Negative			Positive							
14												Positive						
15													Negative					N
16														Negative				
17															Acid			
18						Negative												N
19																		N
20																		
21						Acid 10 days												
23									Ac									N
24																		N
25									Ac									
26																		N
	Motile	Negative	Negative	Negative	Positive													

Note: A = Alkaline
Ac = Acid
N = Neutral

Three strains did not ferment xylose even after thirty days incubation, and one strain fermented slowly, giving acid after ten days incubation. With the exceptions mentioned the strains tested conform very closely to what are considered the typical cultural characteristics of typhoid.

Serological Studies:

The immune serum was prepared by inoculating healthy adult rabbits intravenously at seven day intervals with a suspension of organisms killed by heating to 60°C. for one hour, followed by two or three doses of living organisms. Eight sera were prepared and the titre in each case determined by agglutination tests using the homologous organism. The titre in the different sera varied from 1-500 to 1-15,000. In all tests the bacterial emulsions were made to uniform turbidity, using a twenty-four hour agar slant culture, The tests were set up in .4cc quantities, equal parts of serum dilution and bacterial emulsions. These were incubated for two hours at 37.5°C., readings taken and then kept at ice box temperature over night after which a second reading was taken. Each of the twenty-five organisms was agglutinated against each of the sera prepared. Table III is a summary of the results obtained.

		2 1-15,000		3 1-12,000		20 1-3000		7 1-3000		8 1-3,000		5 1-1,000	
	Strains	Titer	Reaction	Titer	Reaction	Titer	Reaction	Titer	Reaction	Titer	Reaction	Titer	Reaction
I.	1	1/100	4+	1/100	+-	1/2000	4+	1/500	3+	1/3000	4+	#	-
	4	1/100	+-	1/100	+-	1/4000	3+	1/500	4+	1/3000	3+	#	-
	6	1/100	+-	1/100	+-	1/2000	3+	1/500	3+	1/2000	3+	#	-
	7	1/100	2+	1/100	3+	1/3000	3+	1/3000	4+	1/2000	3+	#	-
	8	#	-	#	-	1/2000	3+	1/3000	3+	1/3000	4+	#	-
	20	#	-	#	-	1/3000	3+	1/500	3+	1/3000	4+	#	-
	21			#	-	1/2000	3+	1/2000	3+			#	-
	23			1/200	3+	1/1000	3+	1/1000	3+			#	-
	24			1/100	2+	1/3000	4+	1/3000	3+			#	-
	25			1/200	3+	1/3000	4+	1/1000	3+			#	-
	26			1/100	3+	1/3000	4+	1/3000	3+			#	-
II.	11	1/200	4+	1/100	4+	1/500	3+	1/1000	3+	1/2000	4+	#	-
	9	1/1000	3+	1/1000	3+	1/3000	3+	1/2000	3+	1/3000	3+	#	-
	12	1/1000	3+	1/1000	3+	1/2000	4+	1/2000	3+	1/1000	3+	#	-
	10	1/200	3+	1/1000	3+	1/2000	3+	1/500	3+	1/1000	4+	#	-
	13	1/200	4+	1/200	3+	1/2000	3+	1/200	3+	1/1000	3+	#	-
	14	1/200	3+	1/1000	3+	1/1000	3+	1/1000	3+	1/3000	3+	#	-
	15	1/100	3+	#	-	1/2000	3+	1/2000	3+	1/300	4+	#	-
	16	1/100	2+	1/100	4+	1/3000	3+	1/500	3+	1/3000	4+	#	-
	17	1/100	2+	#	-	1/300	3+	1/1000	3+	1/3000	3+	#	-
	18	1/1000	3+	1/1000	3+	1/2000	3+	1/1000	3+	1/300	4+	#	-
	19	1/100	2+	1/100	+-	1/1000	3+	1/1000	3+	1/2000	3+	#	-
III	2	1/15000	3+	1/12000	3+	#	+-	#	+-	#	-	#	-
	3	1/15000	3+	1/12000	3+	#	+-	#	+-	#	-	#	-
IV	5	#	-	#	-	#	-			#	-	1/1000	3+

Note: # = No agglutination at 1/50 or below

Discussion:

From Table II it will be seen that it would be possible to group our strains into three types according to the xylose fermentations. That is, Type I, 21 strains giving acid in xylose; Type II, three strains fermenting xylose slowly or not at all; Type III, #5, xylose negative, slightly motile,"blue"typhoid
This classification would correspond to the one given by Weiss[3]. His findings for the non-fermentation of xylose by the Rawlings strain paralleled ours. Teague[5] objects to such a classification on the ground that the so-called negative strains are not really incapable of fermenting xylose but ferment it slowly. His strains all showed acid after eight days. By plating xylose negative strains on solid medium containing xylose he was able to pick daughter colonies which showed acid earlier than the original colony. His strains however, were grown in a xylose containing medium for several generations. No attempt was made by the author to discover xylose positive mutants from the xylose negative strains but the xylose tubes in the cases mentioned showed no acid after thirty days whereas the xylose positive strains with the exception of #21 showed abundant acid in twenty-four hours.

The salacin fermentation seemed variable and did not correlate with any other characteristics. Our strains were uniformly negative in dulcite and arabinose. Teague[5] reports eleven out of forty-one strains fermenting these sugars slowly.

In litmus milk it will be noted that only seven strains followed the usual classificatinn in turning back to neutral after two or three weeks. The others with the exception of #5 gave about the same degree of acidity after one month as they did after twenty-four hours. #5 in dextrose, lactose, mannite and saccharose was perfectly typical but in litmus milk it gave a deep blue color in one week, it shows only slight blackening with lead acetate agar and slight darkening in 2% peptone gelatine. It is sluggishly motile and quite inagglutinable.

The need for some classification of the antigenic types of typhoid became apparent from the results obtained in running routine agglutination tests on organisms isolated from clinical cases of typhoid and from some Widals. Parke Davis antityphoid serum, serum from the city laboratory at Wichita and serum sent us from the University of Chicago was used for checking up the antigenic properties of the following organisms; 1, 2, 4, 6, 20, 50, 51, 52,. Culturally they were all typhoid. #50, 51, 52 were all strains isolated from feces in cases resembling influenza. These strains unfortunately were lost. The following chart shows the results of these tests.

TABLE IV.

NO.	Parke Davis		Wichita		Univ. of Chicago	
	Titre	Reaction	Titer	Reaction	Titer	Reaction
1	1/50	_	1/50	-	1/8000	4+
2	1/10000	3+	1/50	1+	1/10000	4+
4	1/1000	4+	1/400	4+	1/2000	4+
6	1/2000	4+	1/400	4+	1/4000	4+
50	1/50	-	1/50	-	1/50	-
51	1/50	-	1/50	-	1/50	-
52	1/50	-	1/50	-	1/50	-
20	1/4000	4+	****	****	1/8000	4+

In running Widals in this laboratory it is customary to set up each serum with the following organisms: B. typhosus, Paratyphosus A and B. In such a Widal giving negative with the strain used, #2, the serum was again set up using three other strains of typhoid. It again gave nagative with #2 but was strongly positive with the other two strains. Such antigenic differences might constitute an important source of error in making routine laboratory tests.

Numerous observers have remarked upon the antigenic differences in typhoid. Durham[6] observed such differences but did not attempt to group his strains. Weiss[3] found that his series of organisms could be divided into three types, each distinct from the others. His results parallel ours very closely.

It has been customary to think of typhoid as being quite homogeneous but so many observers have recorded the finding of poorly agglutinating strains or quite non-agglutinable ones that it is obvious that some classification of these variants could be made. It seems hardly logical to call such strains atypical typhoids as they may be quite typical culturally. Colonel Russel* suggested that in typing typhoid, strains isolated from the blood stream only should be used because of the doubtful etiological relationship of feces strains to the disease and the tendency of feces and sewage strains to variation. He cited Hiss'"collection of odds and ends of typhoid" isolated chiefly from the feces of cases and carriers. This collection was made up of typhoid strains differing in some respect from the accepted standard.

If further work in this laboratory and others should confirm this typing of typhoid it might prove the value of a polyvalent rather than a monovalent typhoid vaccine.

From Tables III and IV it will be seen that the strains of typhoid used differ greatly in their agglutinating properties. Type I is made up of eleven organism whose immune serum agglutinates all other organisms in Type I, in dilutions practically as high as that given for the homologous organism. Type I serum also agglutinates Type II organisms but in lower dilutions, conversely the Type I organisms are agglutinated by Type II serum but in lower dilutions than are the Type II organisms. These two groups are closely related and inter-agglutinate to a marked degree. Types I and II serum give slight or no agglutination with organisms in Types III and IV. Type III consisting of two strains #2 and #3 interagglutinate perfectly at 1-15,000 but this high titred serum agglutinates members of Types I and II only in low dilutions or not at all. Only one organism is included in Type IV, namely #5; this organism agglutinates only with it's homologous serum which had a titer of 1/1000. #5 serum does not agglutinate any of the other strains at any dilution. These results are shown graphically in Tables V and VI.

Inagglutinable strains of typhoid have been reported previously from time to time. Williams[7] reports the isolation of non-agglutinable strains from sewage. Weiss[3] reports the isolation of two non-agglutinable organisms

TABLE V.

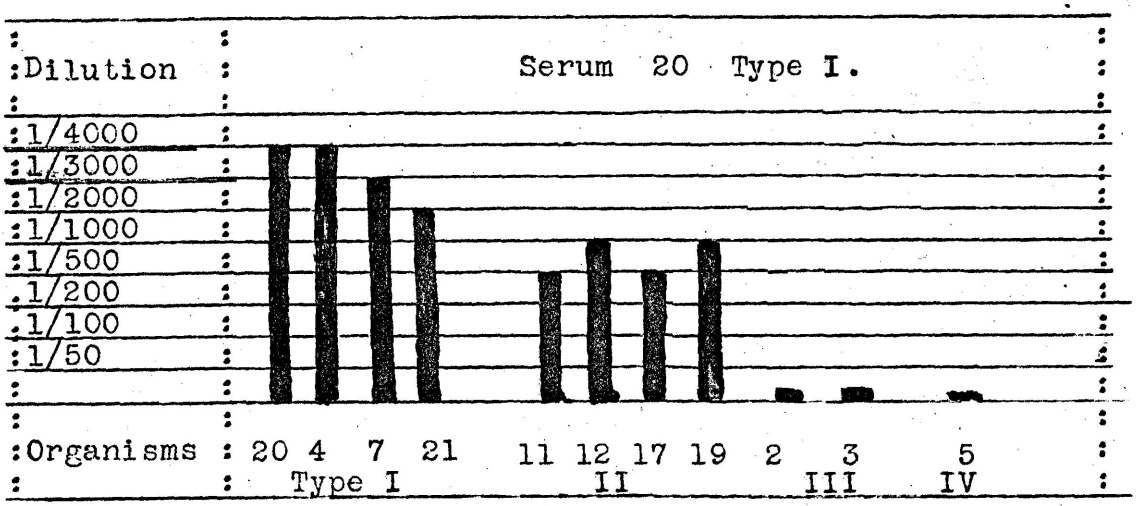
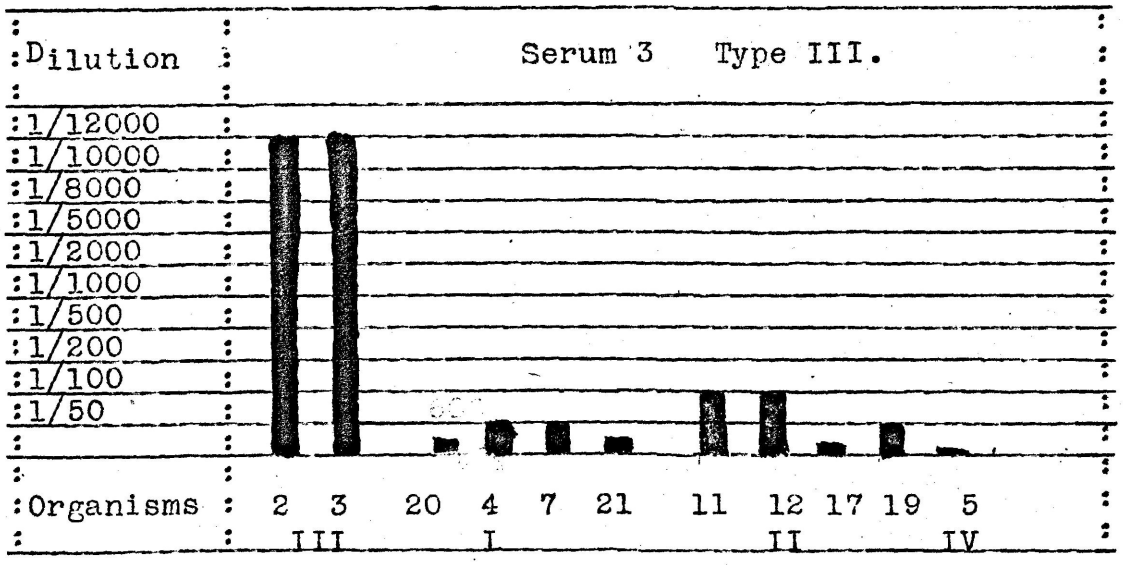


TABLE VI.



from the stools of normal individuals in addition to his "Plosser" strain of which he does not give the history. Our non-agglutinable strain was isolated from the stool of a case resembling paratyphoid in its explosive onset. The organisms were very numerous on the plates made from the feces sample. The patients blood after three weeks did not give a Widal with the strain isolated nor with any of our laboratory strains. The case ran a protracted typhoid-like course. It might be suggested that such non-agglutinable strains belong to an heterogenous group agglutinating with the homologous serum only and not giving cross agglutination, corresponding to Type IV pneumococcus.

The author expects to extend this work to include agglutination test with a larger number of organisms and other sera and also check it up by means of absorptions tests.

Summary:

The twenty-five strains of typhoid used gave typical cultural reactions in the common media, four of them were variable in xylose giving a negative reaction. One of these strains was a "blue" typhoid and sluggishly motile.

Antigenically the strains fell into four groups, the mahority are included in Types I and II which interagglutinate in low dilutions but in the higher dilutions give agglutination only with the corresponding type. Type III interagglutinated slightly or not at all with the other types and Type IV is a non-agglutinable strain.

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* Colonel Russel. local citation.